

Mechanotransductive regulation of gap-junction activity between MLO-Y4 osteocyte-like and MC3T3-E1 osteoblast-like cells in Three-Dimensional Co-Culture.

C.M. Juran¹, E.A. Blaber¹, E.A.C. Almeida¹. ¹Space Biosciences Division, NASA Ames Research Center, Moffett Field, CA, USA

Cell and animal studies conducted onboard the International Space Station and formerly on Shuttle flights have provided groundbreaking data illuminating the deleterious biological response of bone to mechanical unloading. However the intercellular communicative mechanisms associated with the regulation of bone synthesis and bone resorption cells are still largely unknown. Connexin-43 (CX43), a gap junction protein, is hypothesized to play a significant role in osteoblast and osteocyte signaling. The purpose of this investigation was to evaluate within a novel three-dimensional microenvironment how the osteocyte-osteoblast gap-junction expression changes when cultures are exposed to exaggerated mechanical load.

MLO-Y4 osteocyte-like cells were cultured on a 3D-Biotek polystyrene insert and placed in direct contact with an MC3T3-E1 pre-osteoblast co-cultured monolayer and exposed to 48 h of mechanical stimulation (pulsatile fluid flow (PFF) or monolayer cyclic stretch (MCS)) then evaluated for viability, proliferation, metabolism, and CX43 expression. Mono-cultured MLO-Y4 and MC3T3-E1 control experiments were conducted under PFF and MCS stimulation to observe how strain application stimuli (PFF – cell membrane shear or MCS – cell focal adhesion/attachment loading) initiates different signaling pathways or downstream regulatory controls. Total/Live cell count, viability and metabolic reduction (Trypan Blue, LIVE/Dead and Alamar Blue analysis respectively) indicate that mechanical activation of MC3T3-E1 cells inhibits proliferation while maintaining an average 1.04×10^4 % reduction/cell metabolic rate, $*p < 0.05$ $n=4$. MLO-Y4's in monolayer culture increase in number when exposed to MCS loading but the percent of live cells within the population is low (46.3% total count, $*p < 0.05$ $n=4$), these results may indicate an apoptotic signaling cascade. PFF stimulation of the three-dimensional co-cultures elicits a universal increase in CX43 in MLO-Y4 and MC3T3-E1 cells, illustrated by immunohistological observation. Increased CX43 expression is also observed with the three-dimensional co-cultures with MC3T3-E1 MCS stimulation but the increased gap-junction protein presence was limited to the osteoblast-osteocyte interface region. Previously reported PCR evaluation of osteogenic markers further corroborate that the co-cultured population's communicative networks play a role in translating mechanical signals to molecular messaging. These findings suggests an osteocyte-osteoblast gap-junction signaling feedback mechanism may regulate mechanotransduction of apoptosis initiation and transcription of cytokine signaling proteins responsible for stem cell niche recruitment much more directly than previously believed.

These investigations were supported by a NASA Postdoctoral Program (NPP) Fellowship to Cassandra M. Juran.